

**REMARKS/ARGUMENTS**

Claims 11, 12, 17-19 and 21 are pending. For convenience, the Examiner's rejections are addressed in the order presented in an March 3, 2005 Office Action. New claims 29-31 are added.

**I. Status of the claims**

New claims 29-31 are added and are directed to Mkinase proteins that have kinase activity. Support for these amendments is found throughout the specification, for example, at page 52, line 18 and Figure 5. These amendments add no new matter.

**II. Rejections under 35 U.S.C. §101**

Claims 11, 12, 17-19 and 21 are rejected under 35 U.S.C. §101 as allegedly lacking a specific and substantial utility. Applicants continue to assert that the claimed Mkinase nucleic acids encoding Mkinase proteins have utility as diagnostic agents to detect cancer. Applicants refer the Examiner to exhibits and arguments made in a response filed on December 9, 2004. As the Exhibits and arguments were entered, they are not repeated in this response.

Applicants respectfully assert that the Mkinase protein has the additional specific and substantial utility of binding to TRAF4 protein. This property of the Mkinase protein can be used for, *e.g.*, TRAF4 detection or purification. TRAF4, as is well known, is a member of the tumor necrosis factor receptor factor family. TRAF4 expression is observed in, *e.g.*, breast carcinomas. *See, e.g.*, specification at page 4, line 31 through page 5, line 5. TRAF proteins are known to regulate CD40 signaling through TRAF binding sites. *See, e.g.*, specification at page 5, lines 6-9.

Binding of Mkinase to TRAF4 is asserted throughout the specification, for example, at page 4, line 21; page 27, lines 20-22; and at page 33, lines 16-20. Attachment of Mkinase to a solid support to isolate binding partners, *e.g.*, TRAF4, is asserted at, *e.g.*, page 27, line 24 through page 28, line 10. Labeling of Mkinase to allow detection of binding partners is asserted at, *e.g.*, page 19, lines 30 through page 20, line 6 and at page 28, lines 25-31.

The application, therefore, provides at least two asserted utilities for the Mkinase protein. Mkinase proteins have utility on their own because of the association of the encoding chromosomal Mkinase gene with certain cancers. This utility is described in detail in previously submitted response. In addition, the encoded Mkinase protein has the useful property of binding to the TRAF4 protein. TRAF4 has been reported to overexpressed in certain cancers. *See, e.g.*, specification at page 4, line 31 through page 5, line 5 and references cited therein. On reading the specification, one of skill in the art would recognize these asserted utilities of Mkinase proteins. In view of the asserted Mkinase utilities, withdrawal of the rejections under 35 U.S.C. §101 is respectfully requested.

### **III. Rejections under 35 U.S.C. §112, first paragraph, enablement**

Claims 11, 12, 17-19 and 21 are rejected under 35 U.S.C. §112, first paragraph as allegedly lacking enablement. Specifically, the Office Action alleges that the claimed invention is not supported by either a specific and substantial asserted utility or a well-established utility, and that because of the alleged lack of utility, one of skill would not know how to use the claimed invention.

Applicants have submitted arguments in support of the Mkinase utility asserted in the application as filed in Section I of this response. In view of those arguments, Applicants respectfully request that the related rejection under 35 U.S.C. §112, first paragraph also be withdrawn.

Claims 11, 12, 17-19 and 21 are also rejected under 35 U.S.C. §112, first paragraph as allegedly lacking enablement for proteins that are not identical to SEQ ID NO:2. Specifically, the Office Action alleges that undue experimentation is required to practice the invention. In response, Applicants again assert that undue experimentation is not required to practice the claimed invention, including the newly added claims 29-31.

The specification provides a very detailed description of the amino acid sequence of the Mkinase protein, *e.g.*, SEQ ID NO:2. Functional assays to identify Mkinase proteins of the invention are disclosed in the specification. For example, the specification describes

methods of assaying Mkinase binding to proteins such as TRAF4 and methods of assaying Mkinase kinase activity. *See, e.g.*, Examples 2 and 5. Therefore, the specification provides examples of how to screen for Mkinase binding and kinase activity. Moreover, with respect to the kinase activity specifically recited in new claims 29-31, regions of the Mkinase protein that share homology with conserved domains from known kinase proteins are identified, *e.g.*, at Figure 7. One of skill could use the alignments at Figure 7 to determine which Mkinase residues could be modified, while still maintaining protein function, *e.g.*, kinase activity.

In order to establish a prima facie case of lack of enablement, the Examiner has the burden to establish a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). The Examiner has not done so. As the Office Action recognizes, inoperative embodiments can be encompassed by the claims. *See, e.g.*, Office Action at page 6. The Examiner has not established that those of skill, having the specific Mkinase structure of SEQ ID NO:2 and the guidance of the specification to determine the function of Mkinase proteins with 95% identity to that sequence, would be unable to practice the claimed invention without undue experimentation. In order to identify Mkinase proteins with 95% identity to SEQ ID NO:2, those of skill are able to manipulate amino acid sequences, using recombinant DNA techniques, such as PCR, that have been available for nearly two decades. Moreover, systematic, large scale mutagenesis of proteins is commercially available, from *e.g.*, Geneart, GmbH of Rengensberg Germany. *See, e.g.*, Appendix A. The binding and kinase assays described in the specification and used to determine activity of Mkinase variants are also routine and well within the ability of those of skill. Thus, any experimentation required to practice the claimed invention is not undue.

Applicants respectfully bring to the Examiner's attention two recent decisions by the Board of Patent Appeals and Interferences: *Ex parte Sun*, Appeal No. 2003-1993 and *Ex parte Bandman*, Appeal No. 2004-2319. In both cases, the board found that claims directed to sequences with 80% or 95% identity to a reference sequence were enabled because the supporting specifications provided a single reference sequence and an assay for activity of the encoded protein.

In view of the above amendment and arguments, Applicants, respectfully request withdrawal of the rejection for alleged lack of enablement.

#### **IV. Rejections under 35 U.S.C. §112, first paragraph, written description**

Claims 10, 11, 17-19 and 21 are rejected under 35 U.S.C. §112, first paragraph for allegedly failing to comply with the written description requirement. Applicants respectfully bring to the Examiner's attention that claim 10 is already cancelled. According to the Office Action, the recited binding function is not described. To the extent the rejection applies to the amended claims, Applicants respectfully traverse the rejection.

The Office Action also appears to assert that the genus of Mkinase proteins is not described in the specification. *See, e.g.*, Office Action at page 7. However, as currently applied, the specification does comply with US patent law for description of a nucleic acid or amino acid sequence. The Federal Circuit court of Appeals addressed the description adequate to show one of skill that the inventors were in possession of a claimed genus at the time of filing. *See, e.g., Enzo Biochem, Inc. v. Gen-Probe, Inc.*, 63 USPQ2d 1609 (Fed. Cir. 2002). An applicant may also show that an invention is complete by

... disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention ... *i.e.*, complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. *Id.* at 1613.

Furthermore, "description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces." *See, e.g.*, 66 Fed. Reg. 1099, 1106 (2001).

As indicated above, the specification provides a very detailed description of the amino acid sequence of the Mkinase protein, *e.g.*, SEQ ID NO:2. Assays to identify functional Mkinase that bind to proteins such as TRAF4 or that have kinase activity are disclosed. *See, e.g.*, Examples 2 and 5. With regard to the Office Action's assertion that "binding is not

sufficient to describe a genus", Applicants respectfully point out that the disclosed Mkinase binding partners were selected from hundreds of thousands of non-binding proteins and thus, that binding is a definitive characteristic of Mkinase. In addition, new claims 29-31 now recite that the Mkinase protein has kinase activity, as is demonstrated in the application at Figure 5 and Example 2. The specification also provides alignments of the Mkinase kinase domain with kinase domains from known kinase proteins at Figure 7.

The Examiner has failed to provide any reason why a skilled person, who is aware of the disclosed specific amino acid sequence of Mkinase and the disclosed assays of Mkinase function, would be unable to recognize that the Applicants invented the claimed invention, including proteins with at least 95% identity to SEQ ID NO:2.

Applicants again bring to the Examiner's attention the *Sun* and *Bandman* decisions by the Board of Patent Appeals and Interferences. In both cases, the board found that claims directed to sequences with 80% or 95% identity to a reference sequence were described because the supporting specifications provided a single reference sequence, teachings of areas of the claimed sequences that could be modified, and a functional assay for activity of the encoded proteins. Such teachings are included in the present application, as indicated above.

Applicants also direct the Examiner's attention to Example 14 of the Synopsis of Application of Written Description Guidelines which analyzes a claim directed to a protein having an amino acid sequence at least 95% identical to SEQ ID NO:3 and that has a specific activity. In these Guidelines, the Patent Office concluded that the claim was adequately described within the meaning of 35 U.S.C. §112, first paragraph. The Mkinase protein does have kinase activity as demonstrated in Figure 5 and Example 2. Therefore, on the basis of Written Description Guidelines issued by the USPTO, the present claims directed to sequences that are 95% identical to SEQ ID NO:2, meet the written description requirement.

In view of the above amendments and arguments, Applicants, respectfully request withdrawal of the rejection for alleged lack of written description.

Appl. No. 10/088,961  
Amdt. dated July 5, 2005  
Amendment under 37 CFR 1.116 Expedited Procedure  
Examining Group 1646

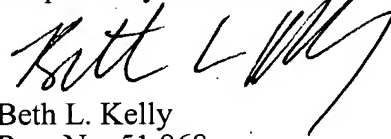
PATENT

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



Beth L. Kelly  
Reg. No. 51,868

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**DNA SEQUENCING**

Standard Sequencing  
BAC Sequencing  
Pricelist

**Gene Variants** [print](#)

(few defined variants)

*test several purely rational protein variants*

	50	60	70	80
<u>wildtype</u>	GVVPILVELDGVNCHKE	SVSCEGEGDATY	GKLTILKFICT	
H56A	GVVPILVELDGVNCHKE	SVSCEGEGDATY	GKLTILKFICT	
K57A	GVVPILVELDGVNCHKE	SVSCEGEGDATY	GKLTILKFICT	
F58A	GVVPILVELDGVNCHKE	SVSCEGEGDATY	GKLTILKFICT	
S59A	GVVPILVELDGVNCHKE	SVSCEGEGDATY	GKLTILKFICT	
V60A	GVVPILVELDGVNCHKE	SVSCEGEGDATY	GKLTILKFICT	
H56A d67-70	GVVPILVELDGVNCHKE	SVSCEGEG	----GKLTILKFICT	
K57A d67-70	GVVPILVELDGVNCHKE	SVSCEGEG	----GKLTILKFICT	
F58A d67-70	GVVPILVELDGVNCHKE	SVSCEGEG	----GKLTILKFICT	
S59A d67-70	GVVPILVELDGVNCHKE	SVSCEGEG	----GKLTILKFICT	
V60A d67-70	GVVPILVELDGVNCHKE	SVSCEGEG	----GKLTILKFICT	

**The Problem:**

Functional protein analysis often requires the generation of a number of variants of the wildtype gene. Alanine- or cysteine scan, glycosylation modification, deletion experiments are just a few examples. To date, this required tedious mutagenesis bench work. PCR based protocols involve the screening of many clones to identify non-wildtype and correct mutants and are error prone on the insert and vector integrity. This is time consuming, and can become expensive not only on the sequencing cost level.

**The Solution:**

Save your valuable time and budgets for the really important experiments and profit from GENEART's extended service. Based on our expertise in *de novo* gene synthesis, we can generate gene variants with complex combinations of substitutions, insertions and deletions with delivery times starting at two weeks.

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**APPENDIX A**

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when you know which few sites you should permute

	50	60	70	80
<b>Library</b>	.....	.....	.....	.....
clone A01	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT
clone A02	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT
clone A03	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT
clone A04	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT
clone A05	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT
clone A06	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT
clone A07	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT
clone A08	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT
clone H12	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT	GVVPILVELDGVNCHQSVSGEGEGDATGKLTLDICT

**The Problem:**

Directed evolution strategies are perhaps the most efficient methods of creating proteins with improved or novel properties. Until recently, conventional fragmentation-based DNA shuffling protocols or error-prone PCR were the methods of choice to create suitable libraries for *in vitro* evolution assays. However, these strategies either depend on the availability of highly homologous genes or create huge random libraries with a vast amount of non-functional variants.

**The Solution:**

*De novo* library synthesis allows for a true rational design that is based on available information about the protein and offers a maximum of flexibility. While leaving most of the coding framework untouched and accurate, it is possible to degenerate only those protein positions that are likely or known to contribute significantly to the protein's function.

**Deliverables:**

Degenerated libraries can be delivered in different formats:

- **non-amplified library:** Up to  $10^{11}$  molecules as verified by real-time PCR.
- **amplified library:** 5 µg of linear DNA ready to clone via 5' and 3' restriction sites.
- **cloned library:** 10 µg of plasmid DNA. Library subcloned into customer's vector. Includes glycerol stock of total library transformants.

**Quality control:**

Amplified and cloned library pools will be bulk sequenced.

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Additionally, a peer group of 48, 96 or 192 individual transformants will be sequence verified. The statistical analysis of the obtained data includes:

- **determination of correctness** of the non-degenerated part of the sequence
- **even distribution of nucleotides** at the degenerated sites
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(# of codons x 20 variants)

*when you don't know which site(s) you have to permutate*

	50	60	70	80
<u>wildtype</u>	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT
H56X	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT
K57X	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT
F58X	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT
S59X	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT
V60X	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT
S61X	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT
G62X	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT
E63X	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT
G64X	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT
E65X	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT	GVVPILVELDGDVNGHKF SVSCEGEGDIATYCKLTILKEICT

**The Problem:**

Often, proteins need to be optimized by directed evolution without specific knowledge about the molecule. Random mutagenesis libraries of such genes create only a tiny fraction of all possible variants, rendering successful screenings unlikely or suboptimal.

**The Solution:**

A systematic approach to improve a protein's performance is sequential permutation. It resembles an alanine scan but replaces each amino acid by all 20 possible amino acids simultaneously. For each codon, a small site-saturated library is constructed, that can be delivered as a pool or separated for all 19 substitution variants. This strategy scans the whole (or part of the) protein to create a map of neutral, beneficial and detrimental amino acids for each position.

**Deliverables:**

All gene variants are subcloned into the customer's vector and delivered as 10 µg of plasmid DNA. Depending on the details of the subsequent screening assay, site-saturated constructs can be delivered in two different formats for each codon:

- mixed (small library containing all 20 variants)
- separate (each of the 19 variants will be delivered separately)

**Quality control:**

All of the separated variants will be 100 % sequence verified. A mixed site-saturated library will be bulk sequenced for each codon.

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